

Poster Abstracts

CONFERENCE PROCEEDINGS

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Subtropical Agriculture and Environments Society

Venue: South Texas College, 400 N. Border, Weslaco, TX 78596

F Building, Student Activities and Cafeteria

Date: February 8, 2019

Animal Science

AS1

Evaluating the effects of cyclic monensin feeding on VFA in ruminally-cannulated steers consuming a low-quality forage.

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Monensin has been observed to alter VFA production by causing a reduction in the acetate:propionate ratio which allows more energy availability to the host. However, monensin has been observed to lose these beneficial effects after prolonged continuous feeding. The objective of this study was to determine if feeding monensin cyclically, rather than continuously, can prolong its beneficial effects. Twelve ruminally-cannulated steers fed a low-quality forage (LQF; 4.9%CP) diet were randomly assigned to one of three treatments: 1) control (CON; 0 mg·hd⁻¹·d⁻¹ monensin), 2) monensin (MON; 200 mg·hd⁻¹·d⁻¹ monensin) or 3) cyclic (CYC; 200 mg·hd⁻¹·d⁻¹ monensin for 14 d and 0 mg·hd⁻¹·d⁻¹ monensin for subsequent 14 d). Dried distillers grains (1 kg·hd⁻¹·d⁻¹) were fed for monensin inclusion. Each of the four periods were 28 days in length. Rumen fluid was collected with a suction strainer 0, 2, 4, 8, and 12 h after feeding on d 14 for VFA analysis. Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with terms in the model including treatment, hour, and treatment×hour with animal as a random effect. Periods were analyzed separately because of a period effect ($P \leq 0.01$). In Period 1, a treatment×hour interaction was observed for propionate ($P \leq 0.01$) and the acetate:propionate ratio ($P \leq 0.01$); a treatment effect was observed for acetate ($P \leq 0.01$); and an hour effect was observed for acetate ($P \leq 0.01$). In Period 2, a treatment effect was observed for propionate ($P \leq 0.01$) and the acetate:propionate ratio ($P \leq 0.01$) and an hour effect was observed for acetate ($P \leq 0.01$), propionate ($P \leq 0.01$), and the acetate:propionate ratio ($P \leq 0.01$). Periods 3 and 4 will be analyzed next to determine the effects of cyclic monensin feeding.

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AS2

Alternative method of assessing cattle temperament

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Common methods of evaluating cattle temperament in a restrained environment are chute exit velocity (EV) and chute scores (CS) while pen scores (PS) evaluate the cattle in an unrestrained environment. The challenge with CS and PS is that they are subjective measurements. The objective of the project was to determine if a range finder could be a suitable method of objectively assessing cattle temperament while the animals were in a pasture, an unrestrained environment. Mature cows ($n = 19$) of mixed breeds were utilized. The strongest correlations were between PS and EV ($r = 0.70$) and PS and the initial distance a human could walk to the cows in a pasture before they moved ($r = 0.59$). All other correlations had correlation values less than 0.45. Evaluating restrained assessment methods to predict unrestrained assessment methods stepwise regressions were utilized. The highest R^2 ($R^2 = 0.47$) was when exit velocity was the independent variable with PS as the dependent variable. Stepwise regression was also used to determine if any of the unrestrained pasture assessments could predict the unrestrained assessment of PS. The highest R^2 ($R^2 = 0.31$) was when initial distance a human could walk to a cow in a pasture before they moved was the independent variable with PS as the dependent variable. Combining correlation and regression analysis, using a range finder to determine the distance a human approaches a cow in a pasture before the cow moves away could potentially be an objective measurement than could be utilized instead of the subjective PS.

AS3

Addition of sodium nitrite to sawdust used for smoke generation when smoking meat products increased the depth of the smoke ring in beef steaks.

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New York Strip Steaks (*longissimus lumborum*; $n = 6$) were cooked then were either not smoked (NNC), smoked with no cure salt (SNC) or smoked with cure salt (SSC). The smoking measurement process followed a 6 to 1 ratio, for every 6 parts of sawdust there was 0.0005 lbs of water. For SSC, 25 ppm of cure salt was added to the measurement ratio. SNC and SSC steaks were smoked for 2 hours on an Oyster Electric Plate, the container was covered on top with aluminum. Each steak was cooked on a George Foreman Grill for 15 mins and allowed 5 mins to cool down. After cooking, color measurements were taken with a Miniscan EZ from left, right, and center. The data was analyzed using GLM procedures of SAS. Addition of sodium nitrite to sawdust increased ($P < 0.05$) the depth of the smoke ring, and had no influence ($P > 0.05$) on the external color of the cooked steaks.

AS4

Characterization of voltage sensitive sodium channel gene of *Haemaphysalis longicornis* (Acari: Ixodidae), a new invasive tick species in the United States

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Haemaphysalis longicornis (Neumann), the Asian longhorned tick, is a three-host tick species that originates from temperate and subtropical areas of East Asia where it infests and transmits zoonotic pathogens to livestock and humans. *H. longicornis* was detected in the United States outside of quarantine for the first time on August 2017 infesting a sheep in New Jersey (NJ). Since then, this invasive tick has been detected in Arkansas, Connecticut, Maryland, New York, North Carolina, Pennsylvania, Virginia, and West Virginia. Treatment of livestock with acaricidal drugs has been the main strategy to control ticks. However, the development of acaricide resistance needs to be anticipated. Acaricide resistance is an inherited phenotypic trait frequently conferred by mutations in the pesticide's target sites. Pyrethroid resistance in several arthropod species can be caused by conserved mutations in the voltage sensitive sodium channel gene (*vssc*). In order to detect future development of pyrethroid resistance in *H. longicornis* using molecular techniques, our objective was to characterize the *vssc* gene. RNA was obtained from a pool of *H. longicornis* collected in NJ. Degenerate primers were used to amplify two conserved segments of the *vssc* gene and a 3'/5' rapid amplification of cDNA ends approach was used to get the full-length transcript. The translated amino acid sequences and the putative protein secondary structure was compared to the homologs *vssc* of the tropical cattle tick, *Rhipicephalus microplus* (Canestrini), the brown dog tick, *Rhipicephalus sanguineus* (Latreille) and other parasitic arthropods. No mutations previously associated to pyrethroid resistance were detected in the NJ ALT samples analyzed. To the best of our knowledge, this is the first characterization of a gene in *H. longicornis* associated with acaricide resistance.

AS5

Integrated Control of Cattle Fever Ticks on Cattle Outside of the Quarantine Zone

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The southern cattle fever tick (CFT), *Rhipicephalus microplus*, has been eradicated from the U.S. since 1943. However in Mexico, CFT are endemic and there are outbreaks in the U.S. along the U.S./Mexico border every year. More than a million head of cattle are brought into the U.S. from Mexico every year. They are inspected at the border to make sure that they are tick free. Any ticks found on the cattle result in a refusal of the herd and sent back to Mexico. CFT are one of the most economically important vector of disease in cattle costing the cattle industry worldwide billions. *R. microplus* are vectors for the protozoan parasites *Babesia bigemina* and *B. bovis* where the reintroduction of them into the U.S. herds could lead to mortality rates as high as 90%. About 50% of Mexican cattle coming in the U.S. could be asymptomatic carriers of the disease. As a prevention, the USDA-APHIS regulation is that Mexican cattle are restricted from areas where the vector could be. This project consists of blood samples taken from cattle that are from Fredericksburg, Texas and brought to the USDA-ARS Cattle Fever Tick Research Laboratory. Blood samples were taken from the cattle where gDNA was extracted. A nested PCR method was utilized to test for *Babesia bigemina* and *B. bovis*. All samples have yielded negative results, demonstrating that the current preventative measures are working.

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Understanding biological control of ticks; using the brown dog tick, *Rhipicephalus sanguineus* Latreille, as a model system for cattle fever tick, *Rhipicephalus microplus* (Cannestrini)

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The cattle fever tick, *Rhipicephalus microplus* is an exotic livestock pest in south Texas and northeastern Mexico. Although it has been eradicated in the U.S. it frequently re-invades from Mexico via stray livestock and alternate wildlife hosts. Classical biological control of *R. microplus* using tick parasitic wasps, *Ixodiphagous* spp (Hymenoptera: Encyrtidae) from its native range in Southeast Asia may enhance eradication efforts since biological control insects could target cattle fever ticks on hosts such as nilgai and white-tailed deer that are difficult to treat with conventional pest management strategies. To better understand how to rear and evaluate the parasitic wasps from Asia, we are studying *Rhipicephalus sanguineus* which is a close relative of the *R. microplus* and is known to be attacked in Texas by *Ixodiphagous hookeri* Howard. This species of parasitic wasp that lays its eggs inside *R. sanguineus* nymphs and adult wasps emerge from engorged nymphs. We are following methods from a similar study in Brazil which found high levels of parasitism. In our study, *R. sanguineus* of all life stages were collected between May 2018 to January 2019 from dogs at the Palm Valley Animal Shelter (McAllen, TX). The numbers of tick nymphs and adults were recorded, and ticks were placed in vials and held at 27°C and 70% humidity for emergence of wasps. To date no wasps have been collected, which is surprising given that *I. hookeri* was originally described from Corpus Christi, TX. We plan to continue collections of ticks through May 2019. These techniques will be used to develop rearing techniques and evaluate host range of the Asian *Ixodiphagous* species.

AS7

Field testing of the remotely operated sprayer for cattle fever tick on nilgai

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Abstract. The southern cattle fever tick, *Rhipicephalus microplus* (Cannestrini) is an exotic livestock pest in south Texas and northeastern Mexico. Although it has been eradicated in the U.S. it frequently re-invades from Mexico via stray livestock and alternate wildlife hosts. Nilgai antelope, native to India and established in the rangelands of South Texas and northeast Mexico. They are highly mobile hosts of cattle fever ticks and implicated in the widespread movement of this serious livestock pest. Nilgai are also highly valued by sportsmen and provide considerable income for ranchers and wildlife managers. A remotely activated sprayer has been developed by ARS for use on ranches with nilgai infested with cattle fever ticks. The sprayer is activated when nilgai cross under established fence crossings. *Steinernema riobrave*, an entomopathogenic nematode, originally discovered by ARS and now in commercial production is used in the remotely activated sprayer to control the ticks on the nilgai. A beta test of the nilgai sprayers was conducted from June to August 2018 at a ranch in Willacy County where 12 prototypes were set up and observed weekly. Several mechanical and biological issues were documented, and this information was used to modify the final prototype of the sprayer. A large-scale field test of 150 nilgai sprayers is currently being conducted in Cameron and Willacy Counties.

AS8

Evaluation of *Steinernema riobrave* nematodes for control of the southern cattle fever tick, *Rhipicephalus microplus*

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Steinernema riobrave Cabanillas, Poinar, and Raulston are entomopathogenic nematodes native to the Rio Grande Valley of Texas that are being evaluated for eradication of the southern cattle fever tick, *Rhipicephalus microplus* (Cannestrini) on cattle and alternate wildlife hosts such as nilgai antelope.

Entomopathogenic nematodes are microscopic roundworms show promise for control of cattle fever ticks, especially in environmentally sensitive areas because they are non-toxic to vertebrates and native to counties in South Texas where this pest is invasive. In previous laboratory and barn trials, *S. riobrave* was found to be effective for control of *R. microplus*. In this study, we evaluated laboratory reared *Steinernema riobrave*, and a commercial formulation Nemasys-R, (BASF, Raleigh, NC) under field conditions to determine their efficacy against, *R. microplus* on infested cattle. Cattle were maintained in covered pen at the Cattle Fever Tick Research Laboratory, Edinburg, Texas and fed with customized feed and water. We adhered to protocols for the care and use of animals as required by USDA-ARS Institutional Animal Care and Use Committee (IACUC). The facilities are fully accredited by the American Association of Laboratory Animal Care. In each experiment, three cattle received the nematode treatment and three were left as untreated controls. The nematodes were applied in a water solution using a backpack sprayer. Treated animals received 5 liters of the nematode solution. Following application of the nematodes, ticks were counted on each of the infested cattle every two days over a three week period to determine efficacy. We found a significant difference between treated and untreated populations of the ticks on the infested cattle for both the laboratory and commercial formulation of *S. riobrave* which shows promise for use of entomopathogenic nematodes for eradication of this cattle pest.

AS9

White-tailed deer flavor preference for evaluation of novel methods of cattle fever tick control in south Texas

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Cattle fever ticks (CFT; *Rhipicephalus annulatus* and *R. microplus*) can be host to *Babesia bovis* and *B. bigemina*, the causative agents of cattle tick fever (bovine babesiosis). Novel methods are being evaluated to control the spread of CFT on white-tailed deer. In this preliminary study, seven flavoring agents (Essential Caramel Toffee Essence™, Essential Long 'N Lush Essence™, Essential Anise Essence™, Essential Power Punch Essence™, Essential Cover Up Essence™, Essential Mineral Boost Essence™, and Essential Garlic Essence™; Triple E Essentials, Inc.) were evaluated to determine white-tailed deer preference. The preferred flavoring agent will serve as a flavor enhancer for a custom-made feed with an insect growth regulator (IGR) that will be evaluated for efficacy against CFT growth and development in subsequent work. Seven white-tailed deer, housed at Texas A&M University-Kingsville, were used in a 7×7 Latin Square design. Periods were four days in length allowing 3 d for offering of treatment and 1 d for washout. Animals were provided their maintenance ration, Record Rack Deer and Elk Feed® (Sportsman's Choice) and alfalfa, *ad libitum* at 0600 daily. At 1000 daily on d 1-3 of each period, 113.4 g of maintenance ration mixed with the respective flavoring agent was offered to each animal. After one hour, orts were measured to determine total consumption. Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with term in the model including treatment, with animal and period as random effects. No treatment effect ($P=0.19$) was observed for consumption. Numerically, Essential Power Punch Essence™ was preferred (average 0.09 g DM consumption) and will be utilized as the flavoring agent for the IGR feed in subsequent studies.

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AS10

Evaluation of land cover type use by nilgai antelope (*Boselaphus tragocamelus*) in rangelands to assess cattle fever tick dispersal

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Cattle-fever ticks (CFT), *Rhipicephalus microplus* and *R. annulatus*, are vectors of the protozoan parasites responsible for bovine babesiosis (BB), an economically important disease in the livestock industry. Although cattle are the preferred host, wild ungulates, including nilgai antelope (*Boselaphus tragocamelus*), can be infested too, potentially contributing to the spread of CFT and BB. Nilgai, an exotic species native to India, Pakistan, and Nepal, were first introduced to South Texas in 1924. Populations since have increased and are presently widespread throughout South Texas. In the borderlands of South Texas, wildlife can move freely to and from Mexico, where CFT and BB remain endemic, which increases the risk for disease transmission in the U.S. cattle herd. Nilgai are a major concern as CFT hosts due to their large home range and ability to make long-distance movements. With the frequent invasion of CFT, understanding nilgai movement and habitat selection will provide key information needed to eradicate this vector and prevent introduction of this serious livestock disease. Thus, the impact of land cover type on the movement and distribution of nilgai on coastal rangelands will be assessed in the study presented here. The objectives of this research include: (1) quantify land cover within nilgai home ranges, and (2) assess the spatial and temporal distribution of nilgai in relation to vegetation type cover. Using GPS locations from 16 collared nilgai combined with remotely sensed imagery, we will classify land cover and identify areas used by nilgai over the course of a year. These data will help identify focal areas for targeted treatment of nilgai against CFT infestation.

AS11

Genetic structure and diversity of nilgai antelope in Texas

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Nilgai antelope (*Boselaphus tragocamelus*) were released onto private ranches in Texas during the 1920's - 40's. Nilgai now occur throughout much of coastal South Texas and northern Mexico, with an estimate of over 30,000 nilgai in Texas. Although nilgai were introduced over 80 years ago, little is known about nilgai ecology and especially nilgai population genetics in either Texas or their native range in India, Nepal, and Pakistan. We are currently genotyping nilgai tissue samples collected from Kleberg, Kenedy, Cameron, and Willacy counties in Texas, geographically representing the U.S. nilgai range. The goal of this study was to identify genetic markers that will amplify in nilgai to enable fine-scale analyses of genetic structure. We screened 20 microsatellite DNA loci developed for livestock and cervids, and found 7 loci that amplify and have enough variation to identify individuals. The mean number of alleles per locus was 6.14, expected heterozygosity = 0.706, polymorphic information content = 0.666, identity non-exclusion probability 2.6×10^{-7} , and sibling identity non-exclusion probability = 2.6×10^{-3} . We will analyze population structure to infer movements and connectivity, and also determine if founder effect has affected genetic diversity. Finally, we are evaluating the markers for use on fecal DNA in a mark-recapture study on latrine ecology, or repeated defecation at a localized site. The results of this study, the first population genetics analysis of nilgai, will serve as a reference for future genetic studies and provide information for nilgai population management in South Texas.

Environmental Science

ENV12

Fenceline Ecology: Wildlife Use of Fence Crossings in South Texas

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In South Texas, land is primarily used as rangelands, with net-wire fencing being the most common fence type throughout the region. Many wildlife species are incapable of passing over fences. As an alternative, these species use fence crossings, which are areas of the fence where an animal has pushed up or dug underneath the bottom wire. Crossings are beneficial for the movement of wildlife species and population connectivity. However, wildlife that use these crossings to travel between ranches can spread diseases or parasites. For example, in South Texas, the spread of Cattle Fever Ticks (*Rhipicephalus annulatus* and *R. microplus*) from Mexico is becoming a problem in the cattle industry and a challenge for the Cattle Fever Tick Eradication Program. These ticks, along with other wildlife diseases, can be transferred to new locations via movement of wildlife through fence crossings. There is limited information on type, abundance, or frequency of wildlife species that use fence crossings. We are using remote cameras at 20 fence crossing sites at 2 private ranches in south Texas. We will analyze the visitation and frequencies by wildlife species that used these fence crossings during a 6-month period. Our preliminary results indicate that crossings were used by many species, including but not limited to white-tailed deer (*Odocoileus virginianus*), nilgai antelope (*Boselaphus tragocamelus*), feral hogs (*Sus scrofa*), and coyotes (*Canis*

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latrans). Our results will be important for better understanding the movement of wildlife species which can help prevent the spread of wildlife diseases and disease vectors.

ENV13

Survey of Plant Parasitic Nematodes Associated With Common Weeds In South Texas

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Presence and geographical distributions of three damaging nematode pest (*R. reniformis*, *M. incognita*, *T. semipentrans*) have been recorded only once in the LRGV (Heald, 1970). With year round growing seasons, weed pest are a real problem for many of our growers. With some growers leaving the field fallow until planting season, these places can serve as potential host reservoirs for plant parasitic nematodes (PPN). With the changes in climate and passage of time, a better understanding of the current plant parasitic nematode population in the region could give insight into patterns and persistence of PPN in the RGV. In this study 12 important PPN genus will be assayed in the RGV to determine new and persistent genus in five important agricultural weeds. This study will be used to correlate soil type with specific PPN genus and or persistence.

ENV14

Varying Growth Inhibition by Native South Texas Tree Species on Invasive African Grass

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The hypercompetitive success of three invasive African grasses in South Texas, including Buffelgrass (*Pennisetum ciliare*), Kleberg bluestem (*Dichanthium annulatum*) and Guineagrass (*Megathyrsus maximus*), have greatly diminished ecosystem services and natural regeneration of native plant species and communities. These tropical grasses pose what is arguably the most serious biodiversity conservation issue in the LRGV. Preliminary field observations suggest, however, that several native trees, such as Texas Ebony (*Ebanopsis ebano*) and Huizachillo (*Senegalia schaffneri*), impose significant inhibitory effects on the growth of African grasses and could potentially play a role in vegetation reclamation initiatives. In order to compare, contrast and quantify the potential herbicidal/allelopathic qualities of native trees, an experiment was carried out by transplanting and measuring growth and mortality rates of robust shoots of three African grass species under the canopies of four native tree species, including the two aforementioned trees as well as honey mesquite (*Prosopis glandulosa*) and huizache (*Vachellia farnesiana*). Five replicates of five potted grasses for three invasive grass species were planted in bottomless pots under four different native trees. Differences in mortality rates and growth rates were measured and recorded after six and nine-month growth periods. One-sided T-tests were used to measure statistical significance of differing grass growth results. Mortality rates for all African grasses was greater under huizachillo (100%) than the other three tree species ($p < .0006-.004$), while varying degrees of limited growth and mortality rates were measured under Texas ebony, mesquite

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and huizache trees. Kleberg bluestem grass suffered higher mortality rates than Guineagrass, which may be due to intolerance to shade.

ENV15

Insect-plant interactions in native but invasive silver leaf nightshade (*Solanum elaeagnifolium* Cav.)

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Silver leaf nightshade (*Solanum elaeagnifolium* Cav.) is a herbaceous perennial that causes huge economic loss worldwide. Besides being a host to pathogens and herbivorous insects, it grows as a weed in different cropping systems (e.g., cotton and grain sorghum) and is also poisonous to livestock. The species does extremely well, because it is versatile and can live in poor soil and nutrient conditions. In addition, it also has extraordinary defense mechanisms that include structural defenses such as trichomes and spines, and chemical defenses such as alkaloids and phenolics. And, in addition to sexual reproduction through seeds, it can also regenerate from their rhizomatous roots. Interestingly, the species is native to Southwestern United States, and is widespread in the Lower Rio Grande Valley (LRGV). While the species isn't a huge problem yet in the valley, in near future this could be a major concern. Using multiple populations along the valley, we examined the factors that contribute to its successful growth and establishment, and also if local pests and diseases affect these traits. Our preliminary analyses show that mowing of field during growing seasons significantly induces defenses in the species, and consequently insect herbivores that feed on the species (caterpillars, beetles, and weevils) cause lower damage when compared to unmowed plants. The main herbivores found were Texas potato beetle (*Leptinotarsa texana*), green peach aphid (*Myzus persicae*), tobacco horn worm (*Manduca sexta*, and flower weevil (*Trichobaris texana*). We are currently exploring how herbivores, pollinators, and predators affect multi trophic interactions in the species, and using this as a model to understand insect community dynamics in natural and agricultural ecosystems.

ENV16

Multivariate Comparative Analysis of Eight Tamaulipan Plant Communities

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South Texas occupies a biogeographic transition zone between temperate North America, the Mexican neotropics and Chihuahuan desert, and has consequently been recognized distinctly as the "Tamaulipan brushlands." No comprehensive, comparative study has ever been undertaken, however, on the many and varied plant communities that occur in the region. This study compares and contrasts eight refugial, primary plant communities in the Lower Rio Grande Valley to test the null hypothesis that they comprise to a single definable vegetative type. four 50 m transects were established randomly at each site and all woody plants over 1 m in height were mapped out 10 m from the transect line for a total of 2,000 m² surveyed at each site and 16,000 m² for the study. Plant height, spatial distance from neighboring plants, and canopy cover were recorded to measure frequency, density, dominance, and the importance value of each woody species. Species-area curves estimate the minimum quadrant size necessary to adequately characterize a community. Comparison of species diversity between biotic communities were measured using Shannon and Simpson indexes. Multivariate analysis were conducted with R statistical software using the community ecology package "Vegan." Permutational multiple analysis of variance (PerMANOVA) examine the relationship between plant community composition based on dominance and study sites. Nonmetric multidimensional scaling (NMS) ordination compares the level of similarity of plant communities against one another. Results provide baseline data, which indicate that there are

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distinct plant communities in south Texas that constitute a variety of vegetation types. Further results will correlate plant communities with edaphic factors such as water availability, temperature, and soil type.

ENV17

Examining the role of insect community dynamics in forest restoration success in La Sal Del Rey

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While forest restoration success has been well studied from physiological standpoint, we still lack a clear understanding on how other factors such as insect community dynamics (herbivores, predators and decomposers) affect seedling success and establishment in restoration programs. In addition, recent evidence also suggests that plant success can also be influenced by beneficial microbes such as mycorrhizal fungi. To examine the impact of these two factors in detail, we are currently working on a forest restoration program in the subtropical region of South Texas at in La Sal Del Rey, in collaboration with US Fish and wildlife, and a Dutch reforestation company -Land Life Company. The program has planted approximately 10,000 native tree seedlings in a factorial design with and without mycorrhizal fungi. From the group of 25 species planted, we selected the following species; Berlandiers Fiddlewood, Skeleton-leaf Golden eye, Brasil Bluewood, Huisachillo, Lotebush, Snake-eyes, Coma Saffron Plum, Wright's Acacia to take a closer look at the impact of herbivores and their natural enemies on their success. Our main hypothesis is that these seedlings will considerably vary in their susceptibility towards herbivores and will also induce defenses post herbivory. In addition, we also hypothesize that mycorrhizal fungi will benefit the seedlings in establishment and will provide protection against herbivores. We are collecting data on insect damage, and species associated insect communities using a combination of field observation and trapping methods. We plan to follow up these observations by profiling the physical and chemical defenses of these species using chemical ecology and analytical chemistry methods.

ENV18

Propagation and Characterization of Walker's Manihot, *Manihot walkerae*, an Endangered Species of Wild Cassava

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Walker's manihot (*Manihot walkerae*), a close relative of cultivated cassava (*M. esculenta*) is an endangered species native to the Lower Rio Grande Valley (LRGV) in Texas. Federal and conservation agencies are working on strategies to conserve this species. One effective conservation measure is through reintroduction, which requires better knowledge of the reproductive biology of the species in order to be successful. The overall goal of this research is to screen different genotypes of Walker's Manihot for cold tolerance and cross hybridize cold tolerant genotypes with cassava to design high biomass Cassava germplasm, specifically optimized for food production in areas with cold temperatures. At Texas A&M AgriLife Research in Weslaco, TX we initiated studies to explore efficient propagation methods and for enhancing resilience by expanding genetic diversity of the native *M. walkerae* populations. Stem cuttings and fruits of *M. walkerae* were collected during 2017 and 2018 from several nature parks in the LRGV and San Antonio, Texas and a germplasm collection containing 13 genotypes was established. The germination rate of the seed was very low (5%) and did not increase despite treatments with giberellic acid or heat to the seeds. We also established *in vitro* cultures through embryo rescue and from shoots grown from stem nodes, cultured in Murashige and Skoog (MS) medium, containing 0.113 mg/L 6-Benzylaminopurine (BAP), 0.093 mg/L 1-Naphthaleneacetic acid (NAA), 0.108 mg/L kinetin, and 0.173 mg/L giberellic acid. Root initiation was observed in MS medium with or without growth regulators.

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ENV19

Invasive Species Control with Unmanned Aerial Systems and Herbicide Ballistic Technology

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The diverse and rugged terrain of the Hawaiian Islands requires natural resource managers to use helicopters for surveillance and control of invasive species. Unmanned Aerial Systems (UAS) provide an on-demand platform for remote sensing and have been used for mapping of plants and animals of interest (e.g., invasive and endangered) in wildlife conservation. Herbicide Ballistic Technology (HBT) consists of encapsulated herbicide formulations in soft-gel projectiles delivered by an electro-pneumatic marker and is a valuable tool to treat isolated plant targets during helicopter surveillance operations. We have developed a UAS compatible HBT platform (HBT-UAS) to rapidly deliver pesticides to targets from a UAS. Projectile dispersion was tested from 2 – 10 m from target at 2 m intervals by discharging ten round bursts into a kraft paper target and measuring relative distances of the perforations. The circular error probable (CEP), a circle containing 50% of projectiles, for each test ranged from 1.9 cm to at 2 m from target to a maximum of 5.6 cm at 8 m from target. The HBT-UAS was then tested on three different UAS to determine their suitability for field operations: DJI Spreading Wings S1000+, DJI Matrice 600 Pro, and Yamaha RMAX. The Matrice 600 Pro was determined have an adequate balance of available flight time and autonomous capabilities for field use.

Entomology

ENT20

Effects of Foliar Fertilization of Citrus with Calcium and Silicon on the Bio-ecology of the Asian Citrus Psyllid, *Diaphorina citri*

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Huanglongbing (HLB) is arguably the most destructive citrus disease in the world, and has invaded the United States and Texas in the last decade. HLB is caused by the bacteria *Candidatus Liberibacter asiaticus* (CLas) and is vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (*D. citri*). Management of this incurable disease includes vector control to reduce spread and impact. In commercial groves, vector control is achieved via the use of insecticides. Empirical reports indicate that application of calcium (Ca) and silicon (Si) may interfere with the insect population in many cropping systems. The goal of this study was to evaluate the effect of different formulations of calcium and silicon on the biology and population densities of psyllids on citrus. The effects of calcium, silicon, and their combination were tested on psyllid survival and densities on potted sour orange trees. Greenhouse studies showed foliar applications of calcium silicate and calcium metalosate significantly reduced psyllid survival and population densities by 33% and 19% relative to the untreated control. In subsequent field experiments in a one year-old sweet orange grove, application of calcium, silicon, and their combination resulted in significant reductions of *D. citri* populations. Foliar applications of calcium metalosate, potassium silicate, and their combination, resulted in 55%, 47% and 42% reduction in psyllid-days, respectively,

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relative to the untreated control. Results of these studies clearly indicated application of Ca, Si, or in combination can enhance plant tolerance to psyllid feeding and should be integrated to current *D. citri* management programs.

ENT21

Comparing Mesh Traps against Standard Traps at Assessing the Asian Citrus Psyllid, a Vector for Citrus Greening Disease

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama is the vector of *Candidatus Liberibacter asiaticus* a bacterium that causes Huanglongbing, better known as citrus greening disease. This disease causes undesirable traits in citrus trees which makes their fruit unfit for sale and ultimately causes the death of the tree. Citrus greening disease has no current cure and mitigation is primarily done by control of vector populations. Assessing ACP populations is an important step for preventing the spread and establishment of citrus greening disease in Texas' commercial citrus industry. Currently, standard yellow sticky traps are used to assess ACP populations but other beneficial insects and debris are also captured in the traps. Standard traps modified with a mesh overlay have been shown to successfully capture ACP while avoiding the capture of larger, non-target organisms. In this project, the mesh trap and standard trap methods are compared to see if mesh traps are as successful as non-mesh traps at assessing ACP populations. Data was collected from 17 independent citrus groves where both types of traps were placed side by side in four separate corners (A, B, C, and D) of the grove at similar conditions. Data collected from April 2018 to January 2019 was used to compare the mesh traps against the standard traps at assessing ACP populations. Results show that there was no significant difference between the mesh trap and standard trap method at assessing ACP populations, while qualitative observations show a decrease in non-target organisms captured in the mesh traps.

ENT22

Determining relative infectivity/virulence (= efficacy) of selected entomopathogenic fungi against ACP using spray exposure bioassays

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To incorporate fungal entomopathogens into an integrated pest management program against *Diaphorina citri*, a screening protocol was established to determine infectivity, virulence, and overall mortality of entomopathogenic strains of fungi. By testing various strains against a standardized mycoinsecticide in a controlled setting, a ranking system has been established in which top performing pathogenic strains can be selected for field trials and future potential commercialization and implementation. Potter's spray towers are utilized to deliver a range of doses of viable spores per milliliter on adult *D. citri* in a spray pattern consistent with the spores/hectare observed in real-world spray applications. After a 7 day incubation period at parameters selected to reflect the conditions in the Lower Rio Grande Valley, mortality of the *D. citri* is assessed and the collected data is analyzed against a mycoinsecticide standard, PFR97® (*Isaria fumosorosea*; Apopka97 strain). The resulting data is analyzed in terms of infectivity and

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virulence, providing insight into mortality, rate of infectivity, and susceptibility to infection. This protocol is being adapted for both primary and secondary acquisition, and will elucidate potentially effective strains to be selected for field testing and eventual commercialization and application on citrus across the Lower Rio Grande Valley and United States.

ENT23

Impact of Bactericidal Spray on *Candidatus Liberibacter asiaticus* Acquisition and Transmission by the Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Liviidae)

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Huanglongbing (HLB) or citrus greening is the most destructive disease known to citrus. HLB is associated with the bacterial pathogen *Candidatus Liberibacter asiaticus* (CLas) transmitted to citrus by the Asian citrus psyllid. To date, HLB is an incurable disease, and affected trees gradually decline with significant losses of production. Antimicrobials are being tested against CLas in infected plants. Two bactericides (Oxytetracycline and Streptomycin) have been registered for HLB control in commercial citrus production with the intention of targeting the bacterium *in planta* and cure affected plants. As psyllid vectors acquire and transmit the bacterium during feeding in plant sap, it is plausible that treatment of citrus with systemic bactericides may interfere with CLas uptake, retention, and transmission parameters. The goal of the present study is to evaluate the effects of the bactericide oxytetracycline on *D. citri* biology and CLas vectoring ability. Survival and progeny production of *D. citri* were evaluated on CLas-free potted plants treated with oxytetracycline. Although *D. citri* was not affected by the bactericide treatment, progeny production was significantly reduced on treated plants relative to untreated control. Testing is underway to determine the effects of oxytetracycline treatment on CLas acquisition and transmission by *D. citri*.

ENT24

Developing methods to collect, process, and screen indigenous fungal strains that naturally attack the ACP in the Lower Rio Grande Valley

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The Asian Citrus Psyllid (*Diaphorina citri*) vectors "*Candidatus Liberibacter* spp.", the causative agents of Citrus Greening Disease or Huanglongbing (HLB). Managing the population of psyllids in the Lower Rio Grande Valley is imperative given the high mortality rate of HLB-positive citrus trees. A facet of integrated pest management in development is the use of strains of entomopathogenic fungi for the biological control of *D. citri*. In an attempt to find endemic strains of pathogenic fungi that grow favorably under Lower Rio Grande Valley environmental conditions and naturally infect ACP, psyllid samples were collected from local RV and residential areas, surface sterilized, and plated. Post-mortem fungal samples were isolated and cryostored for later identification. During a field screening, two samples were collected in Pharr, Texas which resulted in the positive identification of *Beauveria* spp. isolates. The samples were sent to a collaborator in Montana who confirmed the ID and began the process of growing the samples in liquid culture and solid substrate fermentation. The isolates were added to the Agricultural Research Service Entomopathogenic Fungal Library (ARSEF) under the names "ACP01" and "ACP02".

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Future steps include implementation of the isolates into applicable bioassays and characterization of the fungus via radial growth plates, UV tolerance, among others

ENT25

Biological Control of the Asian Citrus Psyllid, *Diaphorina citri*, in the Lower Rio Grande Valley of Texas Using the Ectoparasitoid, *Tamarixia radiata*

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Tamarixia radiata Waterston (Hymenoptera: Eulophidae), is a biological control agent of the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), that is being used as a tool to help reduce psyllid populations in urban environments of citrus growing areas in Texas. The USDA APHIS PPQ S&T CPHST Mission Laboratory has developed methods to produce large numbers of the beneficial insects for the biological control of ACP. Releases were made in citrus trees where plant tissue testing positive for HLB has been detected. In 2010, before we began our releases, we were detecting up 43 immature psyllids per flush in residential citrus. Since our biological control releases began, we have seen the populations gradually decline. The latest finds in 2018 indicate we are observing only <4 immature psyllids per flush. This is a reduction of over 90% of the psyllid population.

ENT26

Effects of release method of *Diaphorina citri* and *Tamarixia radiata* in a mass production program

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Infestation of *Tamarixia radiata* rearing cages with viable and reproductively mature *Diaphorina citri* is a critical step in emergence of the generational *T. radiata* adults. While methods involving a passive approach to release, as well as an active-tapping approach have both been employed, we investigated these methods to standardize the infestation protocols for *T. radiata* and *D. citri*. In the first release method (T1), the *D. citri* were released via passive release method while *T. radiata* were released by tapping release method. In the second release method (T2), the *D. citri* were released via the tapping release method and the *T. radiata* were released via passive release method. In the standard control (C), both *T. radiata* and *D. citri* were released passively, with both containers placed on the bottom of the cage. The outcome of the study showed that the T1 method of release was most effective in producing the greatest number of *T. radiata*. Additional test studies will be explored to enhance mass production efforts.

ENT27

Influence of passive release versus tapping release of *Diaphorina citri* and *Tamarixia radiata* on production of adult *T. radiata* in a mass rearing setting

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To determine the difference in production of adult *Tamarixia radiata*, two different methods of introducing *Diaphorina citri* (Asian citrus psyllids) and *T. radiata* into an insect mass rearing project were investigated. A total of 16 cages were set up and monitored over a period of 8 weeks. Cages were divided by method of release of host-pest and parasitoid; 8 cages were infested using a passive release method and the other 8 were infested using a tap release method. Production and collection of *T. radiata* was recorded daily. The outcome of the study showed that there was an increase in *T. radiata* collected from cages that used the tap release method. These results indicate that there may be a higher mortality rate of psyllids when passive release method is used leading to lower production of *T. radiata*.

ENT28

Mortality of Differing *Tamarixia radiata* Populations in Collecting Jars in Cold Storage

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Efficient storage of *Tamarixia radiata* is important to the production and shipping of the parasitoid insect rearing process. This study focused on longevity of differing populations of the parasitoid in collecting jars held in cold storage. Four different populations of *T. radiata* were placed in wide mouth collection jars – one of two hundred, three hundred, four hundred, and five hundred insects – with a paper square saturated with a solution of honey, water, and Bravo[®] (an anti-fungal agent) for feeding. The vials were then periodically checked, wherein dead insects would be counted and removed from the jars, and the food source square would be rehydrated with the honey solution. Within the first week of storage, rates of mortality were fairly low. However, at the one week mark, there was a dramatic increase in the number of deaths across all collecting jars. Percentage rates of deaths were relatively the same among all vials, with the highest population vial actually having the lowest percentage rate of deaths among all vials.

ENT29

Premature Insect Mortality During “Passive Release” Infestation Method

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A study was conducted to determine the premature insect mortality associated with the “Passive Release” method during *Tamarixia radiata* and *Diaphorina citri* infestations in a mass production program conducted at the USDA Mission Laboratory. After observing eighteen bug dorms over a two week period, it was noted that, on multiple instances, a statistically significant amount of either *T. radiata* or *D. citri* died in their release container before they could properly infest the host plants. Approximately half of the infestations of either species were completed with no premature insect mortality. However, it was noted that almost half of the infestations of either species did see early insect mortality of up to 10% of the studied insects.

ENT30

Methods for host management of *Murraya paniculata* in a *Tamarixia radiata* greenhouse rearing cycle using *Diaphorina citri*, vector of Citrus Greening

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Mitigation of the Citrus Greening disease threatening citrus industry greatly involves suppressing vectors while research progresses for breeding resistances. While commercial citrus applies on-farm pest management, controlling surrounding urban areas, especially in distributed, non-coordinated, and populated locations raises asymmetrical challenges due particularly to the vector's mobility. The United States Department of Agriculture (USDA) implements a successful biological control project to produce and release *Tamarixia radiata*, a beneficial insect that hunts and parasitizes nymphs of the vector, *Diaphorina citri* (ACP), thus reducing successful reproduction. The goal is to maximize controlled *T. radiata* rearing by parasitizing ACP hosted on *Murraya paniculata*. This display outlines the biological control rearing cycle in four phases relative to host plant management as employed at the USDA Mission Laboratory in Edinburg, Texas. The Preparation (P) phase optimizes new growth in *M. paniculata* as a substrate for ACP, selecting plants based on stage, soil fertility, and health; applying favorable conditions for vegetative growth; and trimming buds to initiate flushing. The Infestation (I) phase tailors environmental conditions to insect rearing; matches infestation timing and quantity to host resources; monitors production; maintains soil fertility and weeding; collects and prepares *T. radiata* for distribution; and collects ACP for reinfestation. The Recuperation (R) phase applies fertilizer; cleanses plants of honeydew, molds, scales and algae; and stores plants less intensively. The Aphid control (A) phase segregates plants, applies insecticides, disrupts ant colonies, and exposes plants to local fauna to suppress aphids. As the project has matured, the twelve person team collected and distributed $146,000 \pm 8200$ biological control agents per month. Future improvements target production cycle efficiency, plant and soil monitoring, controlling conditions despite seasonality, and pest management.

ENT31

Glandular and non-glandular leaf trichomes negatively affects growth and development in tobacco hornworm (*Manduca sexta*) caterpillars.

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Trichomes are hair-like projections that develop from a plant's epidermis. They serve to protect plants from abiotic stressors like UV damage and cold weather as well as from biotic factors like herbivores.

Trichomes are generally classified as glandular and non – glandular based on their structure, shape and mode of action. The function of these structures in resistance against herbivores has been well studied, but not much attention has been given to the effects of variation in trichome types on the growth and development of herbivores, such as caterpillars. To examine this, we removed trichomes from the leaves of two plant species from the plant family Solanaceae- *Solanum elaeagnifolium* that produces non - glandular trichomes and *Solanum lycopersicum*- a species that predominantly produces glandular trichomes. The removed trichomes were then added to artificial diets to examine their effects on *Manduca sexta*, an herbivore that feeds and develops on both these species. Our results show that while the caterpillars showed no preference for either trichome diet (diet choice test), trichome diets negatively affected their mass and mass gain, and time to pupate, when compared to control diets. More specifically, non-glandular trichomes from *S. elaeagnifolium* caused more damage than the glandular diet. Based on

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our results, we speculate that although trichomes are considered a first line of defense, they can also cause prolonged and continuous developmental effects in a trichome-type manner, and should be explored further across multiple families and species.

ENT32

Evaluation of temperature and aeration on the longevity of *Steinernema riobrave* in infective juvenile nematodes in a water solution.

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Steinernema riobrave (Cabanillas, Poinar, and Raulston) is being evaluated as a biological control agent of the cattle fever tick, *Rhipicephalus microplus* (Canestrini). The cattle fever tick transmits a disease known as bovine babesiosis. Cattle fever ticks were eradicated from the U.S., but have recently re-invaded South Texas (Fig 1, A). Nilgai antelope, *Bosephalus tragocamelus* (Pallus) (Fig 1C), are implicated in its long range dispersal especially in Cameron, Willacy, and Kleberg counties in South Texas. Remotely activated sprayers set up at nilgai fence crossings are being evaluated for control of *R. microplus* on nilgai (Fig 1B). A solution of water and *S. riobrave* (Nemasys-R, BASF Corporation, Raleigh, NC) is sprayed on the nilgai as it transits the crossing. This study finds that aeration significantly improves the longevity of *S. riobrave* in a water solution when compared to other application prototypes.

ENT33

Identification of agricultural pests from sticky traps using a non-destructive bulk DNA extraction method

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Insect population monitoring is crucial for the implementation of biological control programs, assessing their effects, and for early detection of new economically important pests. Sticky traps have been used widely to monitor harmful and beneficial insect populations worldwide. Although sticky traps offer a fast and effective insect sampling method, they can delay and hinder identification of the collected insects. In addition, the glue on the sticky traps can damage morphological characters needed for accurate identification. DNA technology allows for fast and accurate identification of insects. DNA extracted from communities of insects has the capacity to identify specimens at species level. The purpose of this study is to develop an efficient, cost-effective and accurate method to identify insects captured on sticky traps. We will be focusing on the identification of three economically important moth species: *T. absoluta*, *H. armigera*, and *C. chalcites*. Several collection methods will be tried. We will be comparing the effects on the use of different type of traps, different sizes, and several different environmental conditions; including temperature and humidity. We will be trying four different DNA extraction methods for large volume samples, and the effect of extracting non-target DNA simultaneously. Several detection methods will be compared; including a real-time assay, a next generation sequencing protocol, and a single nucleotide polymorphism protocol. The efficacy, sensitivity and accuracy of these protocols will be evaluated. This study will provide a new method to identify insects on sticky traps. It will allow for the processing of

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several sticky traps at the same time, reducing costs and saving time during monitoring. In addition, it may improve identification of damaged, immature, and incomplete insect samples.

ENT34

Assessment of *Lycium* spp. Endemic to South Texas as Potential Hosts of *Bactericera cockerelli* and “*Candidatus Liberibacter solanacearum*”

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The potato psyllid, *Bactericera cockerelli*, is the insect vector of the fastidious bacterium “*Candidatus Liberibacter solanacearum*” (Lso) haplotypes A and B, which infect solanaceous crops. This pathosystem is known to affect potato production in the Americas and New Zealand. The major difficulty in controlling this pathosystem in Texas is in not knowing what non-crop plants are sources of the vector and pathogen arriving in potato fields. In South Texas, there are two endemic species of wolfberry that grow widespread in the field, *Lycium berlandieri* and *Lycium carolinianum*. Therefore, we established four cages containing ten plants of each *Lycium* spp. and exposed them for 7 days to their respective treatment (Lso-free, LsoA, LsoB, and No *B. cockerelli*) to determine if these *Lycium* spp. endemic to South Texas are hosts of *B. cockerelli* and Lso as previously investigated for *Lycium barbarum* and *Lycium chinense* in the Pacific Northwest. Entomological observations were made daily to determine insect reproduction and development. Moreover, plant tissue from *L. carolinianum* and *L. berlandieri* were collected per species 28 days post infestation with *B. cockerelli* pertaining to the four treatments mentioned and used for DNA extraction to determine presence and haplotype identity of Lso. Our findings clearly show that the two *Lycium* spp. are feeding and reproductive hosts of *B. cockerelli*, which was able to complete its entire life cycle from eggs to adults as initially found in *L. barbarum* and *chinense*. Interestingly, LsoA and LsoB were individually detected by PCR in both *Lycium* spp. tested here unlike findings obtained for *L. barbarum* and *chinense* in the Pacific Northwest. If these *Lycium* spp. endemic to South Texas indeed act as inoculum sources of Lso in the field it would make the management to control this pathosystem even more complicated for local potato growers.

ENT35

Biological Control of Housefly (*Musca domestica* L.) with Entomopathogenic Fungi

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The housefly, *Musca domestica* L. is a cosmopolitan insect that constitutes a public health problem in urban areas with inadequate sanitary management. Larvae are immersed in their feeding substrate and they are therefore hard to reach; the adults are more accesible targets for control. The objective of the present study is to evaluate entomopathogenic fungi (listed below) prepared as spore powders (dusts), as infective agents against house fly adults. The study was conducted in Saltillo, Mexico, at the facilities of the Universidad Autonoma Agraria Antonio Narro. The experimental treatments (fungal spores/gram) were as follows: the fungi *Metarhizium brunneum*, at concentrations of 1.4×10^8 down to 1.4×10^5 ; *Isaria farinosa*, 5.6×10^8 down to 5.6×10^5 ; and *Beauveria bassiana*, 3×10^8 down to 3×10^5 . One-liter containers were used as cages for fly maintenance. Each cage had ten adult individuals of *M. domestica*. After exposure of the adult insects to the different entomopathogenic fungi in spore powder preparations, high and significant levels of mortality were observed. Most *Beauveria* preparations reached 100% mortality after five days. High concentrations of *M. brunneum* and *I. farinosa* also reached 100% mortality, but

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only after 7 days. These fungi, in particular *B. bassiana*, could be an alternative for the biological control of adult houseflies.

ENT36

Molecular Diagnostic Methods That Reveal Strain ID and Geographic Source for *Ceratitis capitata* (Mediterranean fruit fly)

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The identification of captured Mediterranean fruit flies (*Ceratitis capitata*) is important for conducting effective eradication efforts. This fly has infested areas in the US that include Florida, Texas, and California. It presents threats to many species of fruits including citrus growing areas such as those in the Rio Grande Valley. Methods for reducing these infestations include the use of spraying pesticides and biological control. Trapped material suspected as wild flies undergo morphological inspection for presence of marking dye that is used to track flies from Sterile Insect Technique (SIT) lab colonies and evidence of irradiation damage of males. At times, unmarked sterile flies are also trapped and can present difficulties to diagnosticians. Morphology alone does not always result in accurate determinations when the suspect may be a sterile fly. Additionally, methods that rely on morphology alone cannot identify the geographic source of an infestation. Recently developed molecular tools that rely on mitochondrial and nuclear markers have helped identify whether a captured fly is an unmarked sterile fly or wild fly. Other molecular markers help identify the geographic origin of the outbreak and may improve existing strategies for reducing future outbreaks. We reveal the results of our work on over 200 flies captures in California using these methods.

ENT37

Biocidal effect of the fungi *Beauveria* and *Metarhizium* upon yellow-fever mosquito (*Aedes aegypti*) larvae

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Aedes aegypti females are hematophagous and they have a predilection for human blood. These mosquitoes are vectors of dengue, chikungunya and zika (Morrison et al., 2008). Due to the increase in temperature and climate change, these vectors find more favorable environments, increasing their populations and thus the risk of contracting the diseases they transmit. *Aedes aegypti* develops resistance to chemical insecticides. We need effective control alternatives that are friendly to the environment and other species; an option for this are entomopathogenic fungi. In this research, we used three concentrations (spores/gram) in starch dust of the fungi *Metarhizium brunneum*: T1 = 1.3×10^8 , T2 = 1.3×10^7 , T3 = 1.3×10^6 , and *Beauveria bassiana*, T1 = 1.8×10^8 , T2 = 1.8×10^7 , T3 = 1.8×10^6 applied (35 mg/cup) to groups of 25 larvae in 150 ml. of water. Each fungus was tested against the four larval instars. The highest mortalities were observed at the highest concentrations: 1.3×10^8 spores/gram of *M. brunneum* (100% after 5 days) and 1.8×10^8 spores/gram of *B. bassiana* (100% after 10 days) both in larval stage III. Mortality (and sometimes fungal development) was observed in all instars. The entomopathogenic fungi *Beauveria* and *Metarhizium* could be interesting, environmentally friendly larvicides of *A. aegypti* in its four larval stages. Mosquito larvae are easily targeted with these fungi,

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compared to adults.

ENT38

National Detector Dog Training Center Trains Canines in Mission Lab for Mexican Fruit Fly Larva

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The main focus of the United States Department of Agriculture's National Detector Dog Training Center (NDDTC) is to train detector dog teams to find prohibited agricultural items entering the country. Canines work in the airports, along the border, in cargo areas, and parcel facilities searching for and detecting prohibited products such as apples, oranges, mangoes, pork, and beef products. Canines Bear and Holden are the first dogs to be trained to take it a step further by locating fruit fly larvae inside host fruit, while at the same time ignoring healthy, non-infested fruit. These two canine teams from the NDDTC in Newnan, GA trained at Mission Lab - Moore Air Base developing and testing techniques to detect Mexican Fruit Fly (MFF) larvae in fruit through a three phase program. During the months of March, May and August of 2018, these canine teams worked inside the Arthropod Quarantine (AQ) building, outside on sidewalks, with fruit on a tarp in a citrus orchard on base, and with fruit hanging from trees in different orchard sites across the Lower Rio Grande Valley. Overall, the project goal was to develop the canines as a tool to aid in survey work and border safeguarding by pinpointing MFF larvae inside infested fruit. Based on the proof of concept and validation through proficiency testing during the trainings, an additional canine has been obtained for training as MFF larvae detector dogs with two additional dogs expected in the near future.

ENT39

Strengthening the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera:Tephritidae), Eradication Program with the use of Attract-and-Kill devices

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The Mexican fruit fly, *Anastrepha ludens*, (Loew) (Diptera: Tephritidae) is a serious pest of numerous fruits in its native countries of Mexico and Central America. *Anastrepha ludens* is a pest of regulatory concern whose detection can lead to the establishment of a quarantine that negatively affects the economic viability of citrus production. Very low economic thresholds-including the detection of five wild adult flies within a three-mile radius circle, a wild mated female, or larva/egg found in fruit trigger a quarantine requiring growers to follow costly and strict treatment protocols before fruits can be harvested. To ensure that *A. ludens* does not permanently establish itself in south Texas, a Sterile Insect Technique program (SIT) comprised of releasing irradiated sterile adults has been ongoing for over 30 years. However, sporadic detections of wild *A. ludens* are made in south Texas where unmanaged residential citrus or abandoned citrus groves are intermingled with managed commercial groves. To complement and strengthen the SIT program, an effective 'Attract-and-Kill' (AK) strategy using a device which incorporated lures to attract adult *A. ludens* and an embedded insecticide as a killing agent has been developed and tested for its effectiveness as part of an integrated approach for *A. ludens* management.

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Results from the study demonstrated the AK devices could significantly contribute to *A. ludens* control in south Texas. These devices could be deployed in commercial groves and residential citrus trees and can effectively be integrated into the ongoing control approaches without negatively impacting the environment and biodiversity.

ENT40

Testing DNA extraction procedures for the preservation of larval characteristics using Scanning Electron Microscopy

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The insect family Tephritidae includes many fly species that are significant agricultural pests of fruits and vegetables grown in subtropical regions. When the flies are detected inside fruits they are in an egg or larval life stage and cannot be reliably identified to a species. It is possible to analyze the DNA of the flies to assist in species identification but it is desirable to also retain each larva for subsequent re-examination of its morphology. In previous studies of the Mexican fruit fly, *Anastrepha ludens* (Lowe), we examined the effect of DNA extraction methods on both DNA yield and downstream use of the treated larva carcass in Scanning Electron Microscopy (SEM) analysis. The tested treatments failed to preserve the larvae for SEM analysis. Here we report the results of new experiments that use modified treatments that either excise tissue of the larva midsection prior to DNA isolation or reduce the size of puncture wounds of larvae. The results demonstrate that (i) both methods can yield nucleic acid extractions suitable for molecular analysis; (ii) the larvae with punctures were not suitable for subsequent SEM work; and (iii) the larvae treated with the midsection excision method were suitable for SEM analysis of morphological characters in anterior and posterior parts of the larva.

ENT41

Characterizing Intraspecific Variation of the Internal Transcribed Spacer 1 (ITS1) in the fly *Bactrocera dorsalis*, an Agricultural Pest of Fruits and Vegetables

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Internal transcribed spacer (ITS) DNA regions located between ribosomal subunit genes in nuclear genomes have been successfully used to develop molecular identification protocols for many eukaryotic species. These spacers can evolve at a relatively fast rate which is good for detecting differences between species; and, because of concerted evolution in the rRNA cistron, the within-species or intraspecific variation of ITS sequences is expected to be relatively low. These attributes can facilitate discovery of fixed, diagnostic markers for species identification. Sequencing of the ITS1 region supports identification of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), an invasive pest of fruits and vegetables that is periodically detected and eradicated from California and Florida. Characterizing the ITS1 variability between individuals of this pest is the first step in confirming ITS1 as a suitable DNA target for additional amplification technologies that provide end-point diagnostics. To support that goal, we align and analyze a dataset of 220 *B. dorsalis* ITS1 sequences from GenBank and compare these to over 500 DNA

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sequences generated from flies captured in California over 10 years. The combined data set includes 15 unique ITS1 sequences of the species that are often separated by a single substitution or base insertion. The three most commonly reported ITS1 types in the data set are found in 89% of the sequenced flies. The ITS1 data support the morphological identifications of these flies as *B. dorsalis*.

ENT42

Developing RNA interference tools to control the potato psyllid and zebra chip disease of potatoes

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Potato is one of the world's major agricultural crops with global popularity as a staple food. In the Americas and New Zealand its production has been severely affected by a tuber disorder colloquially known as zebra chip disease, which putative causal agent is exclusively transmitted by the potato psyllid, *Bactericera cockerelli*. Following the successful transmission by the insect vector, the bacteria "*Candidatus Liberibacter solanacearum*" can cause economic hardship for potato growers by reducing yield and quality. Currently, no tolerance or resistance exist for this fastidious bacterium in commercial potato varieties and the management of the insect vector, which relies almost entirely on chemical insecticides, continues to be the most common management strategy available for this pathosystem. Therefore, new strategies to control the insect vector and pathogen transmission are needed for the potato industry elsewhere. Our research group has developed a tissue-specific transcriptome for *B. cockerelli* that is providing us with the possibility of using RNA interference (RNAi) to control this pathosystem by suppressing the expression of specific genes within the insect vector. The goal of this study is to develop and utilize RNAi tools to silence essential insect genes that result in the abnormal development or premature death of this economically important insect vector.

ENT43

Evaluating two LAMP methods for the detection of *Thrips palmi* and *Thrips tabaci*

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Thrips are small insects in the order Thysanoptera. There are approximately 6,000 species of thrips described, and most are considered to be pest species. Currently, seven pest species of thrips are found in the U.S. They have a wide host range; causing scarring and deformities and eventually killing the plant. Thrips are also a primary vector for plant viruses of the genus *Tospovirus*. Thrips have the potential to cause serious damage to the agriculture industry. Thrips sp. are routinely found on plant material entering the U.S. at ports of entry. Morphological identification may be difficult on these interceptions, especially when damaged or at immature life stages. However, molecular diagnostic tools can provide identifications on these types of specimens. Methods for the identification of *Thrips tabaci* and *T. palmi*, two pests present in parts of the U.S., have been published that use Loop-Mediated Isothermal Amplification (LAMP) technology. This technology amplifies DNA under isothermal conditions which allows a very specific and rapid assay. It is important to examine the validity of these techniques prior to implementation. These two identification methods were replicated at the CPHST Mission Laboratory to demonstrate reproducibility and document performance. The purpose of this study was to determine if the experiments could be successfully replicated, to optimize the methods where necessary, and to determine the practical nature of these experiments for a port of entry. In conclusion, one of the methods was successful and optimized to be used as a possible protocol for the detection of *T. palmi* and *T. tabaci*.

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Electric toothbrush: An alternative to tuning fork and bees for Buzz Pollination

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Buzz pollination, where pollinating insects (e.g., Bumble bees, Carpenter bees) use their thoracic muscles to extract pollen from poricidal anthers - is found in approximately 6% of flowering plants. Solanaceae, a major plant family that houses economically important plants such as tomato, potato, eggplant, and peppers, are primarily buzz pollinated. For plant breeding and research programs, insect pollinators are often undependable as controlled buzzers- due to their unpredictable movement and irregular flower visits. Traditionally, a tuning fork is used as an alternative to insect pollinators, but it is expensive, less durable and in many cases- hard to find at the right frequency in a timely manner. To overcome these limitations, we tested the use of inexpensive and easily available electric toothbrush to replace tuning fork, and examined whether varying the duration of buzzing, affects amount of pollen extracted, when compared to the tuning fork and pollinators. We used a native but highly invasive weed *Solanum eleagnifolium* flowers for our experiments and observed the *Xylocopa* spp. (Carpenter bee) for a natural pollinator behavior. Our preliminary results show that electronic toothbrush is a good substitute as it extracts almost similar amount of pollen as of the tuning fork in any given time intervals. More interestingly, we also found that on average, carpenter bees only buzzed individual flowers for less than three seconds, which was enough to collect maximum pollen as found from our fork and toothbrush experiments. We are continuing our experiments to compare both insect and artificial buzzing to understand optimum buzzing time and pollen removal using multiple species of pollinators and host plants.

ENT45

Host Acceptance Problems by *Diaeretiella rapae* (Hymenoptera: Braconidae) in the Banker Plant System

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The "banker" plant system started in the 70s', when Stacy et al. managed to reduce the number of whiteflies with the release of continuously produced parasitoids (*Encarsia formosa*). The "banker plants are plants that continuously produce a beneficial insect and have the facility to establish a continuous source of parasites, usually on a host different to the one that we are interested in controlling. For a system to be successful, preliminary tests have to be carried out with the beneficial insects and the pests to be managed. The objective of this research was to measure the level of acceptance when *D. rapae* adults are offered a different host than the one they emerged from in the banker plant system. Laboratory and greenhouse tests were carried out to measure basic aspects in the breeding of *Diaeretiella rapae*, as well as acceptance tests concomitant to the change of host. The green corn aphid, *Rhopalosiphum maidis* was tested as an alternative host of *D. rapae* that originally emerged from the cabbage aphid, *Brevicoryne brassicae* (the target pest). The wasps obtained from corn aphid (*R. maidis*) were subjected to an acceptance test of the gray aphid (the original host). The test consisted of Petri dishes where pieces of broccoli leaves were placed with 5-10 individuals of gray aphid and another ten boxes with corn aphid. We observed parasitism attempts (a stereotyped response where the female touches the aphid with the antennae, and curved the abdomen under the body, then attempts to "sting" (parasitize) individual aphids. Each test was performed with 10 repetitions (one wasp) each. A mated female wasp was released to boxes with aphids for 5 minutes and the number of aphids that had contact with the wasp (parasitized) was counted. The corn aphid obtained a % 100 acceptance (full parasitism attempts) by females of *D. rapae*,

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while the aphid of the cabbage showed 0% acceptance (no parasitism attempts). The greenhouse test indicated a low acceptance of 12% of *R. maidis*-reared *D. rapae* towards the cabbage aphid under greenhouse conditions. In our tests, there is a change in the acceptance behavior of *D. rapae* towards the original host when it is produced in an alternate host.

ENT46

Phoretic dispersal of *Brevipalpus* Mites on *Anastrepha ludens*

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Over the last 90 years, the viruses that cause *Citrus* Leprosis have spread northward from Argentina to Northern Mexico. The only known mode of transport for these viruses is within their vectors, the mites of the genus *Brevipalpus*. Given their minute size and inability to produce silk for ballooning, the dramatic spread of these viruses warrants explanation. We document phoretic dispersal of *Brevipalpus* mites on fruit flies and measure the frequency with which such behavior occurs in the wild. We also established experiments to tease apart the factors mediating phoretic dispersal.

ENT74

Beneficial microbes enhance defense against insect herbivores in sudan grass (*Sorghum drummondii*)

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Beneficial plant-microbe interactions in the rhizosphere have been found to enhance plant growth and development. Arbuscular mycorrhizal fungi (AMF), a major group among these microbes, improves plant fitness through the establishment of mycorrhizal symbioses. Despite being successfully established in various natural and domesticated study-systems, relatively little is known on whether AMF has cascading effects on plant defense traits. To test this, we planted Sudan grass (*Sorghum drummondii*), a dry land tolerant species, either inoculated with AMF or left as control as a summer cover crop. We hypothesized that AMF will alter plant defense pathways in Sudan grass influencing the attractiveness of the species to beneficial and damaging herbivores, besides other potential benefits for growth and development. Our results suggest that while AMF inoculated plants had significantly better growth and establishment, they also experienced lower initial incidence and damage by the herbivore fall armyworm (*Spodoptera frugiperda*). In addition, our insect community trapping experiment revealed that AMF inoculated *S. drummondii* attracted more beneficial insects (predators and parasitoids) and less number of damaging herbivores. Taken together, our data suggests that AMF treated *S. drummondii*, can positively influence both growth and defense traits, and has the potential to be an excellent cover crop.

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Asian Citrus Psyllid vs. Natural Predators

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The citrus greening disease also known as Huanglongbing (HLB) is caused by the *Candidatus Liberibacter asiaticus* bacteria, carried by the Asian Citrus psyllid (ACP). Symptoms of this disease include, misshaped fruit, bitter flavor, and discolored leaves. These symptoms are harmless to consumers but is affecting the citrus industry in the Lower Rio Grande Valley. Yellow sticky traps have been used to capture the ACP. The information gathered from these traps helps determine if the groves need treatment to remove the ACP. Predators of the ACP can be found on these traps allowing us to gather information to understanding their predator prey dynamics. Today, there is little information about potential use of natural predators of ACP to be used in eradication efforts in the groves. This research aims to provide a list of potential predators found in yellow traps. Preliminary results show that 10 potential predators of ACP found in yellow traps, *Tamaraxia radiata*, *Clubionidae*, *Ectobiidae*, *Formicidae*, *Chrysopidae*, *Pseudomyrmecinae*, *Blattella asahinai*, *Trachelas*, have been found on the traps. The results of this research would help aid in a better understanding of the predator prey dynamic, by including natural predators in ACP eradication efforts. Future work should compare the efficacy of predator-prey in laboratory conditions.

Horticulture

HORT47

Watermelon performance trial in Weslaco, TX

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Watermelon scientifically named *Citrullus Lanatus*, originated in the Kalahari Desert of Africa, they are categorized as fruits and they contain about 92% of water and 6% of sugars. South Texas, more specifically the Rio Grande Valley region, has favorable climate and soil for production of watermelon. The main objective of this study is to evaluate the performance of 16 commercial varieties of watermelon under South Texas. The experiment was conducted at Texas A&M AgriLife Research and Extension Center at Weslaco, TX. The experimental design was randomized complete blocks with 16 commercial varieties, four replicates per entry, five female plants per replicate per entry plus two males, three feet in-row spacing and three feet between plots. The watermelon varieties were transplanted to the field on 80-inch beds with black plastic mulch and drip irrigation on March 14th, 2018. Data collected were yield, length, width, brix, rind width and color of flesh. Yield and brix were compared between the varieties and fruit graded by size. In conclusion, all the watermelon varieties were marketable according to USDA requirements. And the top three varieties were: ORS6042b, ACX 617755 and ORS6151.

HORT48

Tomato production window in South Texas

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Solanum lycopersicum, commonly known as tomato, originated in South America. It is widely grown as a cash crop, and many commercial varieties have been developed to grow at a wide range of environments. South Texas, more specifically the Rio Grande Valley, has favorable climate and soil for tomato production. The aim of this study was to extend the window of production of tomatoes during Spring. The trial was conducted during spring 2018, seven different varieties of tomatoes in open field were transplanted to white plastic mulch at different planting dates (March 5th, March 20th, March 30th). Marketable yield and non-marketable yield data were collected and compared between all the planting dates. The results show that the first planting date (March 5th) produced higher marketable yield in comparison to the second and third planting dates. Biotic and abiotic factors had a role in the results such as high temperatures close to the end of the season causing stress to the plants, and the rising of insect populations, whiteflies and thrips, causing damage to the plants and fruits. The marketable yield of the second and third planting date did not differ, having an average reduction of 46% in marketable yield compared to the first planting date. Late spring planting produced lower yield and quality but may be viable to extend the production season. More data needs to be collected for economic feasibility. In conclusion, early planting dates in the spring can lead to higher tomato yields and quality and using late planting dates can possibly extend tomato production in open field by a week or two.

HORT49

Texas A&M tomato cultivars in South Texas: Evaluation of productivity and fruit quality during Spring 2018.

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South Texas is one of the main vegetable production areas in the State. However, vegetables growing in the subtropical climate of South Texas face challenges due to harsh environmental conditions that often limit crop productivity (e.g. high temperatures, strong winds, and high pressure of insects and diseases). Tomato is one of the top three most consumed vegetable in the U.S and due to its agronomical requirements and South Texas is an ideal production region. Texas imports an estimated 2.4 billion lb/year of tomatoes to meet its market demand. A recent study showed that Texas consumers are requesting a tomato with “vine ripe” flavor, aroma and texture; and are willing to pay a premium for locally produced tomatoes. This represents a great opportunity for local growers to reclaim part of this market in Texas. Our objective is to evaluate and compare seven tomato varieties for yield and fruit quality in open field in three different planting dates using two different plant density. Tomato plants were transplanted to the field in white plastic mulch and drip irrigated. The experiment consisted of seven varieties with six plants per plot, replicated three times, two different plant density (2 ft and 1.5 ft). Within the seven varieties, five were Texas A&M varieties/advanced breeding lines and two were top commercial varieties from Seminis. In conclusion Texas A&M AgriLife varieties and advanced breeding lines have high yield potential and good fruit quality compared to commercial varieties. Early season planting produced higher marketable yields and fruit quality. The variety that produced higher yield was from Texas A&M. No statistical difference in yield was found between plant densities.

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Nondestructive Method for Fruit Quality Estimation in Tomatoes Using Near Infrared Spectroscopy.

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Non-destructive methods to measure fruit quality are very desirable to use on tomato variety improvement. The ratio between acidity and brix are the main components related to the flavor of tomato, serving as markers for defining consumers' acceptance of new varieties into the market. Determination of these two parameters can be very time consuming and usually require taking the sample to an analytical laboratory, juicing the fruit, and then measuring brix using a refractometer, and acidity using a titration technique. In this work we used the Felix 750; a portable near infrared spectrometer, to develop a non-destructive high throughput and in field-based methodology to estimate brix in tomato. Near infrared (NIR) calibration model for Brix was developed by obtaining the spectra of 100 tomato fruits in the wavelength range between 390nm-1100nm, the same fruits were used to measure brix using a refractometer Atago PAL-1. The data collected was inputted in the F750 model builder software to correlate the spectra with their corresponding reference data to create a calibration model. Different calibrations involving different sets of wavelengths were calculated for a higher calibration and optimized prediction of Brix giving a correlation coefficient of 0.88. Now an optimal calibration model for acidity, lycopene and beta-carotene are being developed. The ability to use a nondestructive, high throughput and in field-based methodology to predict fruit quality will improve future studies on tomato variety development.

HORT51

Producing and marketing South Texas tomatoes to the major metropolitan areas in the United States: Probability of Economic Success

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The Rio Grande Valley (RGV) used to have a solid tomato industry. In 1960 the region supplied around 10% of all the tomatoes consumed in the United States (USDA-NASS, 2018). However, due to high pest and virus pressure, in combination with a nascent out of state and international supply, the industry disappeared in the following two decades. Those issues are still present nowadays and foreign competition has become even more challenging since 50% of U.S. fresh fruit and vegetable imports are entering through Texas land ports (CNAS, 2018). Additionally, when Mexico production is off-season, tomatoes from California, Florida, and Canada dominate the market. Nevertheless, the RGV offers certain comparative climatic and geographic advantages, which could lead to a more competitive industry if combined with optimal tomato varieties, production systems, and marketing windows. Thus, this study evaluates the feasibility of several production systems and calculates the probability of economic success (ES) of marketing RGV's tomatoes to the major metropolitan areas in the country. Probabilities of ES are estimated through a stochastic probabilistic approach, which uses Monte Carlo simulation methods to generate production and market scenarios, where yields and prices are serially and spatially correlated. Results showed that a high-tunnel production system offers the greatest chances of ES, followed by net-house during the fall season. However, only the latter would allow RGV producers to compete nationally.

HORT52

Non-ionic Surfactants and Arrested Ear Development in Corn

Poster Abstracts

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Nonionic surfactants are commonly mixed with pesticides to obtain better coverage on the leaf. There are studies showing that spraying NIS products in the pre-tassel stage of corn can cause arrested ear development. Arrested ear development is not a disease, but it can greatly affect yield. We tested different NIS products to determine if specific products can cause arrested ear development. The trial was set up with four NIS products, a non-treated control, and four replications in a randomized complete block design. The corn hybrid was sprayed with the four products at the V14 stage with a backpack boom. Photographs were taken weekly to record any visual differences. Four weeks after application, ears were sampled and measured for length to determine the effect of NIS on arrested ear development.

HORT53

Evaluation of Potential Benefits of Mycorrhizal Fungi in Improving the Quality and Yield of Commercial Aloe Plants

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Arbuscular mycorrhizal fungi (AMF) are naturally occurring soil fungi that are known to form a mutualistic relationship with a majority of plants. These fungi benefit by receiving photosynthetically fixed carbon from the plants. Mycorrhizal fungi are known to offer a great potential for sustainable agriculture. They have been proven to make a significant positive contribution to plant health as well as positively affecting soil quality. Among the benefits arbuscular mycorrhizal fungi provide to plants include increasing nutrient up take, particularly of P and Zn; improving plant access to water; and increasing both pest and soil borne disease resistance in plants. Past research has shown that AMF have the potential to improve the quality of aloe plants and improve nutrient concentration in the leaves. Aim of this study is to analyze the potential benefits of mycorrhizal fungi to improve the yield and quality of aloe plants and to determine the best inoculation method as part of a standard operating procedure. This is an ongoing study and results will be presented at the conference.

HORT54

Effects of cover crops on insect community dynamics in organic farming

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Poster Abstracts

The use of cover crops provides both direct and indirect benefits to agricultural ecosystems. Cash crops sown after cover crops directly benefit from better nutrient availability, increased moisture retention, and improved weed suppression. Although there is some evidence, we still lack a complete understanding on the effects of cover crops as an insect herbivory suppressor, and the mechanisms that mediate these effects, if any. To test this, we planted two commonly used leguminous cover crop species, Sunn hemp, *Crotalaria juncea*, and cowpea, *Vigna unguiculate* in an organic farm in South Texas and observed their effectiveness in providing pest management while also attracting beneficial insects. Data was collected on plant height, insect damage, and plant biomass, in addition to insect community composition in the field. Our preliminary results on cover crops suggest that Sunn hemp performed significantly better in all measures, and continuously showed significantly lower pest damage (caterpillars, true bugs, and aphids) along with a high prevalence of beneficial insects (Coccinellids, wasps and bees) when compared to cowpea. Sunn hemp also improved (higher species richness) the surrounding insect community in the area and thrived in the summer heat without much resource input. Since host location and host choice by insects are primarily mediated by plant volatiles, we are currently examining whether the two cover crop species differ in their volatile profile- both constitutively emitted, and under herbivory. Future experiments also include examining the residual effects of cover crops on the cash crop.

HORT55

Evaluation of Plant Promoter Strength for Genetic Transformation of Citrus

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The lack of genetic diversity in citrus has increased the effects of environmental pressures, pest and diseases felt by these perennial crops. Although citrus has overcome many diseases in the past, none have been as devastating as Huanglongbing (HLB). There is currently no cure for this disease although several approaches have been taken for disease remediation. The only permanent solution to combat HLB is to externally source disease resistant genes to develop HLB resistant plants. In light of the negative public opinion on genetically modified crops, we propose an all plant based system for genetic transformation. In genetic transformation, the promoter region placed before the gene controls how well the gene is expressed. The cauliflower mosaic virus (CaMV) 35S promoter is the most commonly used constitutive promoter for transformation because of its high gene expression. Alternative plant derived promoters must be evaluated for comparable or greater activity than CaMV 35S for an all plant based transformation system. In this experiment we detail a fast, low cost methodology for testing and comparing promoter efficiency. Promoters derived from *Solanum tuberosum* and *Arabidopsis thaliana* were evaluated using transient gene expression in citrus protoplast, via electroporation, of the fluorescent gene *mCherry*. The brightness of the cell under an excitation wavelength is proportional to the activity of the promoter expressing the fluorescent gene. Transformation efficiency was evaluated over the course of 72 hours based on the amount of transformants per microgram of plasmid DNA used.

HORT56

An improved methodology for the PDS-1000/He system using the GUS reporter gene system

Poster Abstracts

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Genetic transformation is an important tool to introduce new genes coding for disease resistance in citrus. The most commonly used plant transformation methods rely on *Agrobacterium tumefaciens*, which is a gram negative soil bacteria and plant pathogen; thereby the transgenic plants produced are considered as regulated articles by the Animal and Plant Health Inspection Services (APHIS). Biolistic transformations utilize gold or tungsten particles coated with DNA to introduce transgenes into monocot and dicot plants. This method involves biophysical DNA transfection method without any use of *Agrobacterium* so the transgenic citrus plants can be produced with limited regulation. The biolistic methodology normally uses spermidine that is unstable which induces variability from one shooting event to another. Furthermore, we observed that the shooting pattern using the recommended Bio-Rad macrocarrier holder producing a distribution pattern where the center of the explant received little particles. In an attempt to obtain more consistent results, our lab tested gold nanoparticles from Seashell Technology and their proprietary DNA coating buffers which do not rely on the use of spermidine. Also, we tested 30 and 80 square mesh screens as microcarrier holders. Our results indicated that the Seashell Technology protocol is superior to the Bio-Rad recommended protocol and that the 80 mesh screens produced more improved shooting patterns.

HORT57

Recombinase mediated marker excision in citrus protoplasts

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Genetic transformation is a powerful tool for introducing new traits into plants, however, to be able to identify the transgenic plants, molecular markers need to be introduced in addition to the gene of interest. These markers are not well seen by consumers, as some of them code for antibiotic and herbicide resistance. This study is part of a larger project and our objective is to determine the efficiency of the enzyme recombinase in excising these molecular markers from transgenic plants using recombinase mediated cassette exchange. The recombinase gene in this project is driven by a promoter induced by dexamethasone or estradiol. Therefore, concentration of inducing chemicals and best temperature for activity of recombinase enzyme needs to be evaluated. To accomplish this objective, we used six plasmid constructs that are engineered in a way that the visual marker gene *mCherry* has a promote split in two with one part upstream of the recombinase cassette (which include the markers) and the other downstream so that only when the recombinase cassette is excised, the *mCherry* gene is expressed. We developed a methodology for a quick screening of marker excision using electroporation of citrus protoplasts. The chemical inducers were added 24h after transformation and expression of *mCherry* was visualized in an inverted microscope under fluorescent light. Results were observed after 6 days of induction and compared with a control plasmid with constitutive promoter. We observed that the excision efficiency of recombinase varies with type of construct used and also with temperature and concentration of inducer. Additional results will be presented.

HORT58

Poster Abstracts

Testing drought tolerance in Carrizo citrus through elevated expression of RhNAC2 and RhEXPA4 genes from rose plant.

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Citrus is one of the most important fruit crops with high commercial and nutritional values planted in tropical and subtropical areas. As population growth accelerates and the demand for water increases due to urban development, water becomes scarce for agriculture. This situation that has a tendency to exacerbate leading to the need of development of plants that are more efficient in the use of water. To address water stress, plants have already acclimatized to several morphological and physiological changes through altered gene expressions. Since roots are the primary source of water uptake to the whole plant any water stress within the microclimate of roots will always send signals inside the plant with the help of phytohormones. Absciscic acid (ABA) is known to be key hormone synthesized in roots during water stress and acts with other genes to reprogram the plant metabolism against stress. So far several genes has been reported for their role in drought conditions. Among them, NAC regulatory proteins, which act as cofactors with the ABA has shown a significant role in signaling stress-related genes in both biotic and abiotic stresses. Expansins are other important cell wall proteins known to serve during water stress related to cell wall expansion and root elongation. Rose plants overexpressing RhNAC2 (NAC factor) and RhEXPA4 (Expansin) genes has led to rose petal expansion during extreme dehydration conditions and also drought tolerance in rose and Arabidopsis. In an attempt to produce drought tolerant Carrizo rootstock, we created transgenic Carrizo plants overexpressing the rose RhNAC2 and RhEXPA4 genes to test for drought tolerance in greenhouse conditions. The plants are in the acclimatization phase and testing for drought tolerance can be done soon.

HORT59

Effects of superabsorbent polymer-soil interaction on plant growth, development, water use efficiency and soil leachate

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Water and nutrient availability are the most limiting factors in crop production. Crop production in South Texas is challenging because of the area's semi-arid climate that requires constant irrigation to increase crop optimum growth and yield. Previous studies show that superabsorbent polymers (SAP) have been used as soil amendment. They absorb water and nutrients and release them slowly in the soil so the nutrients are available for root absorption for increased crop growth, development and yield. A type of SAP (ZEBA®) was used in this research at concentrations used in Europe at Wageningen Plant Research, Vredepeel to study its interaction with south Texas soil, and to determine its optimum concentration for soils in the area. Results indicate that ZEBA mixed with south Texas soils at 2 kg/1000 kg and 5 kg/1000 showed no statistical difference in all growth parameters studied. Also, no difference exists between the two concentrations in leachate volume, water retention capacity, and nitrate concentration of leachate. However, Capsicum annum grown in these soil concentrations of ZEBA had significantly higher chlorophyll synthesis ($P=0.0103$) relative to ambient south Texas soil with or without fertilizer. Also, soil media mixed with ZEBA used significantly less water ($P=0.0158$) as well as less leachate volume ($P=0.0007$) and nitrate concentration in leachate ($P<0.0001$) compared to soils of south Texas.

HORT60

Hydroponic fodder provides higher nutrition to livestock than barley grain feed

Poster Abstracts

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A review of hydroponic germination and growth of barley grain was conducted and a computer interphase of the growth conditions was simulated. The review indicates that results of barley grains germinated and grown in hydroponic trays for one week show that proteins, neutral and acid detergent fibers, some macro and micronutrients, and fresh biomass are significantly different between barley grain feed and hydroponically grown barley fodder ($p=0.0019$). The fodder which includes both green leaves and root mat could be fed to cattle for rapid weight gain because of its nutritional value. Also, this type of fodder has no wastage because cattle feeds on the entire biscuit including root mat which is rich in enzymes, proteins, and starch. It is recommended that small livestock producers adopt the method to increase cattle production.

HORT61

Effects of Super Absorbent Polymer (SAP) on the Growth, Development, Water and Nitrogen Use Efficiency of Tomato (*Lycopersicon esculentum* Mill.) in South Texas Soils.

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In areas of dry climates, water scarcity is an important constraint for food production. The other fundamental factor that limits crop production is availability of nutrients. Balancing the amount of N needed for optimum plant growth while minimizing the nitrogen leaching into surface water remains a major challenge. Super-absorbent polymers (SAPs) are known to be soil conditioners which reduce soil water loss and increase crop yield. Use of SAP's may effectively increase nitrogen use efficiency by minimizing leaching and enhancing nitrogen and water retention. Here, we evaluated total nitrogen and water retention in soils amended with different rates of SAP (0kg of SAP/1000kg of soil without fertilizer (control), 0kg of SAP/1000kg of soil with fertilizer, 0.5kg of SAP/1000kg of soil, 1kg of SAP/1000kg of soil, 1.5kg of SAP/1000kg of soil, 2kg of SAP/1000kg of soil). We determined the effects of the treatments on growth and development of *Lycopersicon esculentum* Mill. These findings indicate that the application of SAP's increased the water holding capacity of the soil. The mean volume of water ($P<0.0001$) and nitrates ($P<0.0001$) retained in the soils amended with different concentration of SAP's are different to that of control. The results also showed that increasing the SAP rate in the soil increased the amount of water and nitrogen retained in the soil which in turn helped for the better growth of the plants. Understanding the influence of SAP will be helpful for agriculture in soils with limited water availability.

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HORT62

Maximizing Nitrogen Fixation Efficiency in South Texas Cover Crops

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Many farms use leguminous cover crops as a nutrient management strategy to reduce their need for nitrogen fertilizer. Biological nitrogen fixation (BNF) depends on a symbiotic relationship between legumes and nitrogen-fixing rhizobia. Under certain conditions despite inoculation with appropriate rhizobial strains, this symbiosis fails to form. Such failure was observed in a 14-acre winter cover crop trial in the Rio Grande Valley of Texas when three legume species (forage pea, crimson clover, and hairy vetch) produced no signs of nodulation and nitrogen fixation. This study examined nitrogen, phosphorus, moisture, and micronutrients as potential causes for the failure and assessed possible interventions to improve nodulation outcomes. Results from a controlled greenhouse study confirm moisture and micronutrient deficiencies as major suspects in the nodulation failure. Nitrogen and phosphorus deficiencies however were not likely causes, nor was mycorrhizal inoculation an effective intervention to improve nodulation results. Further research is underway to pinpoint which of 6 micronutrients (Co, Cu, Mn, Mo, B, Zn) may be most effective for producing nodulation gains and to compare several field inoculation techniques. Lessons from these studies will be shared with regional farmers to help inform sustainable nitrogen management practices and efficient use of cover crops.

Pathology

PATH63

Exploring a Novel Antidote to Citrus Greening: Antibacterial Activities of Symbiotic Fungal Endophytes in *Citrus* and Related Genera (*Rutaceae*)

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Citrus greening disease, a bacterially-based (*Candidatus Liberbacter*), pathogenic infection of commercial *Citrus* species, threatens citrus industries both nationally and internationally. Efforts to control the disease have focused primarily on controlling the psyllid vector, but with little success. Our investigations approach the citrus greening challenge by focusing on the control of bacterial infections instead of the vector. We propose the employment of molecular techniques to detect potential endophytic fungal biocontrol agents in living materials of native and cultivated members of the citrus family (*Rutaceae*), with an aim to eventually test the antimicrobial activities of these fungal mutualists.

Molecular probes have been undertaken to detect fungal genomes in fresh plant tissues of *Citrus*, *Amyris*, *Esenbeckia* and *Xanthoxylum*. We experimented with DNA extraction techniques and found that the initial use of Clorox to clean off extra-foliar fungal contaminants adversely affects our endophytic fungal extractions. Preliminary results indicate thus far that molecular signals of fungal endophytes are present in *Amyris madrensis*, *Xanthoxylum fagara* and *Esenbeckia berlandieri*. Future studies will refine our molecular techniques to adequately sample diverse communities of fungal endophytes. These techniques will eventually be employed to isolate and test the antimicrobial activities of individual fungal strains from the geographical origin of *Citrus* domesticates, Southeast Asia.

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PATH64

Impact of Genetically Engineered Maize (*Zea mays* (L.)) on Aflatoxin Content

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In maize (*Zea mays* (L.)), certain fungi belonging to the *Aspergillus* genus produces a mold referred to as Aflatoxin. Aflatoxins are highly regulated in many stored grains in the US because of its health concerns to humans and livestock when ingested and therefore is of economic importance in the production of corn, wheat, and other cereal crops. Many researchers believe aflatoxin found in maize was responsible by insect damage during the ear development and kernel fill stages. In the late 1990's, *Bacillus thuringiensis* (Bt) maize hybrids were introduced to minimize losses from Lepidopteran insect pests. Previous research has reported variable results in the ability of Bt maize hybrids to reduce aflatoxin contamination. A field trial was conducted in 2018 at TAMUK University farm to evaluate aflatoxin content in various Bt trait packages of maize grown in South Texas. The trial consisted of 12 maize hybrids of varying Bt traits with 4 replications per hybrid. Ten ears were randomly selected and harvested from each plot for analysis. Harvested ears were shelled, ground and tested for aflatoxin content among the various maize hybrids. Results from this trial, indicated significant ($P=0.05$) differences were observed among the hybrids with aflatoxin content ranging from 1 to 1000 ppb.

Aflatoxin production is conducive in regions that experience warm temperate climates such as the southern region of the US. During the duration of this trial, temperatures ranged from 60 to 100 F with the area receiving a total of 6 inches of rainfall.

PATH65

Production of indole acetic acid in phytopathogenic fungi, *Fusarium oxysporum* and *Macrophomina phaseolina*.

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Indole-3-acetic acid (IAA) is known for their role in plant root interactions with microbial partners. Current study was focused on role of IAA as signal for production between endophytic fungus *Fusarium oxysporum* and *Macrophomina phaseolina*. Culture filtrate of the strain contained traces of IAA.

Addition of tryptophan concentration ranging from 100 ppm in culture medium significantly enhanced production of IAA by *Fusarium oxysporum* and *Macrophomina phaseolina*. The strain was cultured in LB broth media containing tryptophan at 1000 PPM at 28°C and 120 rpm for five days. For comparison, endophyte were also cultured in LB broth having no tryptophan. After five days, the culture were harvested and filtered through Whatman No 1 filter paper. The culture filtrate was centrifuged at 3500 rpm/15 min. The centrifuged and filtered sample was injected into a high pressure liquid chromatograph (HPLC, Hewlett Packard model 1100) equipped with a RP-18 (Beckman Ultrasphere) column 150 mm long and 4.6 mm in internal diameter and with a size of particle of 5 μ m. The mobile phase consisted of a 30/70 acetonitrile-phosphate mixture and the detector was adjusted to a wavelength of 220 nm. The HPLC was previously conditioned and calibrated with known concentrations of IAA, TRP and AA. The results obtained show the ability of *Fusarium oxysporum* and *Macrophomina phaseolina* to produce indoleacetic acid from tryptophan and said metabolite is involved in the plant growth promoter capacity.

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PATH66

Inhibitory effect of ethanol extracts of oregano (*Lippia graveolens* Kunth SL) in *Bipolaris cynodontis*

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Because of their benefits to the environment and to the ecosystem, plant extracts play a very important role in agroecology when used as an alternative for the treatment of infectious diseases caused by phytopathogenic organisms in plants with economic interest. In this work the presence of carvacrol and thymol was quantified in the extracts ethanol of oregano (*Lippia graveolens* Kunth SL) by HPLC, determining that the oregano in study is of chemotype carvacrol, with a concentration of 13.2 ppm; under these conditions the presence of thymol was not detected. In addition, the inhibitory effect of ethanol extracts of oregano (*Lippia graveolens* Kunth SL) was assessed. In concentrations of 1, 2, 3.4 and 10% on the fungus *Bipolaris cynodontis*, we presented the 100% inhibition from the concentration to 2%, the antifungal activity was present in the concentration at 1% from day three with a growth of 8%, advancing until day six with a 33% growth. The fungus *B. cynodontis* was characterized morphologically and molecularly by the region ribosomal (ITS1-ITS4), and it is the first report of this species in Mexico. From this work it follows that the extracts of oregano have potential to be employed in sustainable biocontrol strategies caused by the phytopathogen *B. cynodontis*.

PATH67

Exploration of prophages in *Candidatus L. asiaticus* strains in Texas

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Prophages are the DNA sequences that are inserted and integrated into the chromosome of bacteria by infection of bacteriophages (viruses that infect bacteria). Presence of prophages in '*Ca. L. asiaticus*' (CLas), a bacterium associated with Huanglongbing (citrus greening) disease was first reported in Florida. Due to their lytic property, prophages are considered as a potential tool to devise a control strategy for many bacterial diseases including CLas infection. Recently, it was shown that Type 3 prophage in CLas carries a bacterial defense restriction-modification (R-M) system which was speculated to resist Type 1 prophage invasion. CLas strains from China and California harbored various combinations of Type 1 (SC1-like), Type 2 (SC-2 like) and Type 3 prophages. However, strains in Southern China had predominantly a single prophage, either Type 1 only or Type 2 only, but lacked a combination of Type 2 and Type 3. In addition, CLas strains lacking prophages were reported in Japan and China. A total of 506 DNA extracts were prepared from a total of 276 roots, 50 Asian citrus psyllid (ACP), and 180 leaf samples collected from Texas. Of these samples, 284 (176 root, 50 ACP and 58 leaf samples) tested positive for CLas, which were subjected to prophage typing by PCR using specific primers for each prophage type followed by agarose gel electrophoresis. The identity of PCR products obtained with each prophage type-specific primer set was verified by sequencing. The data showed that 90% of CLas-positive root DNA samples were tested positive for both Type 1 and Type 2, while only 9% and 1% were tested positive for Type 1 only and Type 2 only, respectively. CLas-positive ACP DNA showed a similar distribution to that of root DNA where 86% tested positive for both Type 1 and Type 2, 10% tested

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positive for Type 1 only, 4% tested for Type 2 only. On the other hand, leaf DNA showed a different prophage distribution than that of root and ACP DNA; 76% tested positive for both Type 1 and Type 2, 9% tested positive for type 1 only, and 15% tested positive for Type 2 only. Type 3 prophage was not detected in these samples.

PATH68

Recent Outbreaks of Citrus Canker in Texas, Surveys and Eradication Efforts

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Citrus canker caused by *Xanthomonas citri* subsp. *citri*, is a serious bacterial disease that affects citrus worldwide. There are several pathovars and strains reported for this pathogen; the Asiatic A stain (Cancrosis A) is the most widespread and severe form. Citrus canker was reported in South Texas during early 1900s which prompted quarantine and eradication programs by the federal government. The last report of canker incidence was in two trees from Corpus Christi in 1943 and no canker was found in later surveys until recently. In October 2015, a Mexican lime tree growing in a residential property in Rancho Viejo, TX showed typical citrus canker symptoms and the pathogen was confirmed to be A^w strain of *X. citri* subsp. *citri* with a limited host range. On May 20, 2016, two sour orange trees in a city park located in Houston (Harris County) were confirmed to be infected by the A strain. These trees were destroyed and there were no further finds in Harris County. On August 2, 2016 and on May 18, 2018 positive trees were confirmed in Fort Bend and Brazoria Counties. As of December 2018, 246 trees comprising different cultivars located in residential properties of Harris, Fort Bend, and Brazoria Counties were confirmed positive. The confirmatory tests were performed at USDA-APHIS-PPQ CPHST Beltsville Lab. All the positive trees were removed by Texas Department of Agriculture.

PATH69

Susceptibility of Commercial Cotton Varieties (*Gossypium hirsutum*) to Bacterial Blight *Xanthomonas campestris* pv. *malvacearum* (Xcm)

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Cotton production in the South Texas Coastal Bend area is an important textile crop that brings in high revenue for Producers in the area. In recent years, the bacterial disease caused by *Xanthomonas Campestris* pv. *malvacearum* (XCM) has started to re-emerge under favorable conditions. Bacterial blight has the potential to cause high yield losses through defoliation, stem growth, and boll damage. The most effective method of control of bacterial blight of cotton caused by XCM is through variety resistance. The objectives of the studies were to test and determine the effectiveness of four inoculation techniques (Silwet L-77[®] (0.25% v/v) (wounded/ no Silwet L-77[®], wounded/ Silwet L-77[®], not wounded/ no Silwet L-77[®], not wounded/ Silwet L-77[®], control-no treatment) and to evaluate eleven commercial cotton. All studies were conducted at the Texas A&M University – Kingsville greenhouse research facility. Cotton plants for all experiments were inoculated with XCM (prospective race 18) by backpack spray application at a concentration of 10⁶ bacteria ml⁻¹. During the inoculation technique study, plots were rated for disease 7 days post inoculation (DPI), and 7, 14, and 21 DPI for the variety resistance trials. The most effective inoculation techniques were the use of Silwet L-77[®] treatments. The results indicated that all resistant varieties had little to no disease incidence (0-20%), while all susceptible varieties had high disease

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incidence (60%-100%). Further research to confirm these results will be conducted in field trials at the TAMUK university farm.

PATH70

Screening and characterization of zebra chip disease resistance trait among wild *Solanum* species

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Potato (*Solanum tuberosum*), is the fourth most important food crop in the world, after corn, wheat and maize. As demand for both fresh and processed potato products increase, so does the need to protect crop losses from diseases. Zebra chip (ZC), caused by the bacteria *Candidatus Liberibacter solanacearum* (Lso), vectored by the potato-tomato psyllid (*Bactericera cockerelli*), can cause major yield losses in commercial potato crops. Currently, insecticide applications to control the psyllid is the most widely used method to combat infestations and the spread of Lso. Identification of genetic resistance to Lso could lead to new and sustainable approaches to control ZC and reduce the heavy reliance on pesticides. In this project, geographically-diverse wild *Solanum* species were challenged with Lso and screened for tolerance and/or resistance to Lso. Among forty-four accessions screened till date, one demonstrated resistance to Lso, based on phenotypic and molecular characterization. This Lso-resistant accession is useful to further understand the underlying mechanisms responsible for the resistance, and introgressing the trait into commercial potato cultivars.

PATH71

Microbial consortia as a biocontrol agent for chili pepper wilting

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Currently, the trend is sustainable agriculture, which involves changes in management practices and the use of biocontrol microorganisms for diseases. The aim of this work was to identify the causal agents of wilting in chili peppers and to evaluate the control effect of microbial consortia. Samples were collected from serrano peppers with symptoms of wilt in plots of Altamira and González, Tamaulipas. Phytopathogenic fungi were isolated, purified by hyphae tip and subjected to in vitro pathogenicity tests. Six serrano pepper varieties (HS-52, Coloso, HS-44, Centauro, Tampiqueño 74 and Paradise) were used to evaluate the following treatments: a mixture of *Bacillus*, a mixture of *Trichoderma*, microbial extract (metabolites of *Bacillus* and *Trichoderma*), thiabendazole (60%) and absolute control. *Fusarium oxysporum*, *Fusarium solani* and *Phytophthora capsici* were identified as causal agents of chili pepper wilt. Symptoms of yellowing, flower abortion, wilting, root necrosis and premature maturity of fruits, were observed. The disease incidence was statistically different ($p \leq 0.05$) between varieties with *Trichoderma* (10.67%) and thiabendazole (6.83%); the absolute control showed 31.83% incidence. The severity of HS-52, Tampiqueño 74 and Paradise was significantly different with microbial extract (8.33%), *Trichoderma* (11.30%) and Thiabendazole (6.54%), respectively, for its part control with 17.96%. Phytopathogenic fungi *F. oxysporum*, *F. solani* and *P. capsici* are causal agents of chili pepper

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wilting and with the application of *Trichoderma* and microbial extract the effects of the disease are reduced.

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Yeast mixtures in liquid formulation as active ingredients for biocontrol of *Penicillium* sp and Anthracnose (*Colletotrichum gloeosporioides*)

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In *Citrus* spp fruits the most common postharvest diseases are Clear rot, Green and Blue molds (*Penicillium* spp.) and Anthracnose (*Colletotrichum gloeosporioides*). Biological control using microbial antagonists has received a great deal of attention as a promising alternative to chemical control. Among the antagonistic microorganisms, yeasts have a high potential to be effectively used as biological control agents. In this work, microorganisms used belong to the LBI-CBG (Instituto Politécnico Nacional) yeast and filamentous fungi collection. The best performing yeasts (LCBG-03, LCBG-30 and LCBG-Sc4Y3) were selected for testing their compatibility and biocontrol performance against two pathogenic strains of *Penicillium digitatum* (AL-38) and *C. gloeosporioides* (AL-13). Based on the *in vitro* mechanism of action, competence for space and nutrients and percentage of inhibition of radial growth, the best combinations were selected, as well as their initial cell counts. A liquid formulation was set up and tested in terms of yeast viability and antagonistic behavior preservation. Our results demonstrate that not all combination of yeasts are compatible with each other, so those mixtures were discarded. The formulation favored the antagonistic action of the yeast during the competition between the microorganism and increased the antagonistic activity against the disease and allowed the better survival of yeast and also that biocontrol efficacy depended on the fungus being controlled. One of the best mixtures in terms of number of fungi controlled had an efficacy of more than 50 % in terms of radial growth rate, but also in terms of sporulation delay. This work contributes in the search of those compatible yeast combinations aimed to diminish the fungal losses of citrus fruits.

PATH73

Association of two distinct begomoviruses with a disease outbreak in a south Texas okra (*Abelmoschus esculentus* (L.) Moench) field

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Okra (*Abelmoschus esculentus* (L.) Moench) is an important specialty vegetable crop grown in south Texas for fresh and value-added markets. A variety of pests and diseases often limit the full expression of okra yield potential. During fall 2018, an investigation was conducted to determine the potential causal agent(s) of a virus-like disease outbreak on a 12-ac. okra farm located in Hidalgo county, south Texas. Disease incidence peaked at 100%, leading to total crop loss. Symptoms consisted of foliar yellow mosaic, bleaching, and vein-clearing patterns, suggesting *Begomovirus* (family *Geminiviridae*) involvement of. To test this hypothesis, 10 representative leaf samples were collected across the field and DNA extract from each sample was subjected to rolling cycle amplification (RCA) to enrich for circular

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genome targets. The RCA products were used as templates in PCR with generic primer pairs targeting ~600 bp and ~550 bp partial fragments of DNA-A and DNA-B genome segments of begomoviruses. All ten samples yielded DNA-A- and DNA-B-specific amplicons of the expected sizes. The amplicons were cloned individually into pJET1.2 vector and plasmids carrying the correct size inserts were custom sequenced. BLASTN analysis of the sample-specific sequences revealed the occurrence of mixed infections of two bipartite begomoviruses, okra yellow mosaic Mexico virus (OkYMMV) and cotton leaf curl Gezira virus (CLCuGV) in each sample. While OkYMMV has been previously reported from okra in TX, the detection of CLCuGV represent the first record of the virus on okra in TX. Since both are whitefly-transmitted viruses (and not seed transmissible), the results underscore the need for effective integrated management of the whitefly vector(s) and alternative host plants to mitigate disease spread and impact.